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Anti-arthritis effect of methanol extract of leaves of *Abies webbiana* Lindl. in complete Freund's adjuvant-induced arthritis in albino rats

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Abstract---Aim of the study: To investigate Anti-Arthritis activity of Methanolic Extract of *Abies webbiana* Lindl. (MEAW) leaves in complete Freund's adjuvant- induced arthritis in albino rats. Methods: RA was induced in all study groups 2-6 except group 1 by sub-plantar injection of 0.1ml/kg of CFA agent in left hind paw of albino rats. Group 1 demonstrates Normal control. Group 2 serves as Arthritic control and Group 3 illustrates Standard control (Methotrexate 0.5mg/kg). Group 4-6 serves as Test control receiving differing doses of MEAW of 100, 200 & 400mg/kg. Treatment commences after 8th day of CFA induction in a manner of single dose (p.o.) a day for 20 successive days. Meanwhile, invitro parameters of body weight and paw volume were measured & after day 28th hematological, biochemical, anti-oxidant & histopathological parameters were characterized. Results: MEAW demonstrated dose dependent anti-arthritis activity which was evident with decrease in paw volume & increase in body weight when compared to arthritic control group. MEAW (400 & 200mg/kg) exhibited significant ($p < 0.001$) anti-arthritis activity by surging levels of RBC, Hb & equalizing the levels of WBC, Platelets, ESR, CRP & RF. The anti-arthritis activity was also confirmed with the altered biochemical parameters (AST, ALT, ALP & Total Protein Levels) & antioxidant parameters (SOD, MDA & GSH). Methotrexate & MEAW 400mg/kg also prevented joint destruction (Histopathological Analysis). Conclusion: The study findings illustrate that *A. webbiana* Lindl. may be a potential preventive or therapeutic candidate in treatment of inflammation & arthritis.

Keywords---Arthritis, *Abies webbiana* Lindl, complete Freund's adjuvant, Inflammation.

Introduction

Rheumatoid arthritis (RA), an intractable and highly prevalent autoimmune joint disease, is characterized by nonspecific inflammation of peripheral joints and destruction of cartilage and bone with resultant disability. RA is associated with long-term disability, impaired quality of life and a shortened lifespan. Arthritic joints demonstrate chronic inflammation with features of synovial hyperplasia, angiogenesis and mononuclear cell infiltration of the synovial tissue (pannus).^[1] RA is a chronic debilitating autoimmune disorder affecting the entire system occurring predominantly in females rather than males and surges with age. It is most prevailing form of inflammatory arthritis and globally affects up to 1% of the population in industrialized world, with survival rates comparable to coronary artery disease and cancer.^[2] At present, the pathogenesis of RA remains obscure and how to effectively treat and prevent RA has caused widespread clinical attention. Although, the etiology of RA is still not understood, multiple studies have demonstrated that activated inflammatory T cells, B cells and macrophages invading the joint synovium is the cause of joint and cartilage damage.^[3]

Previous research has demonstrated that complete Freund's adjuvant (CFA)-induced arthritis in rat model sharing common features with human rheumatoid arthritis. CFA-induced arthritis shows immediate local inflammatory reaction persist for 3-4 days followed by chronic systemic reaction that persist for even several months.^[4] The advent of several new therapeutic agents has played a major role in the management of RA. Essentially, safer Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) or 'Coxibs' have become available followed by interventions designed specifically to target pathogenic cytokines have reached the clinic. The other classes of anti-inflammatory and analgesic agents include glucocorticoids, opiates and diproqualone, respectively. Though these drugs possess an effective remedy for RA, each drug has its own demerits as side effects like ulcerations, renal and hepatic diseases. The NSAIDs provide only a symptomatic relief but has no significant effect on the underlying disease process. Due to several restrictions and risks of prevailing therapy, people are exploring radical measures to treat the disease.^[5]

Medicinal plants have been proven to be potent and efficacious in the management of RA. The use of medicinal plants provides another alternative approach for the management of RA and currently a number of medicinal plants are under exploration for development of novel drugs. The anti-arthritic potentials of several medicinal plants such as *Zingiber officinale*, *Aloe barbadensis*, *Withania somnifera* Linn have been reported. Thus, it is expedient to exploit the curative efficacy and deleterious actions, if any, of these herbal plants for providing novel and better treatment alternatives with minimal side effects.^[6] In this study, we have tried to investigate the therapeutic potentials of *Abies webbiana* Lindl. in the management of RA.

Abies webbiana Lindl., is commonly known as Indian or Himalayan Silver Fir belonging to Pinaceae family. *A.webbiana* is a large, tall, evergreen tree habituated in Himalayan regions from Kashmir to Assam states in India.^{[7][8]} *A.webbiana* leaves has been reported as antibacterial and antifungal, mast cell stabilizing, anxiolytic, anti-tumour, anti-inflammatory, anti-tussive, female antifertility, febrifuge, anti-spasmodic properties, central nervous system (CNS) depressant actions and are effective against hyperglycaemia, conception, rheumatism and high temperature.^[9] The plant has been traditionally used treatment of various ailments such as mental disorder, bronchitis, pulmonary infections, carminative, antiseptic, asthma, expectorant, stomachic, cough and decongestant.^[10] Natural monomers or extracts isolated from plants or herbs have shown efficacy in treating various diseases with relatively low toxicity.^[1] It has been reported that flavonoids, Steroids^[11] and Tannins^[12] from extracts of *A.webbiana* are potent anti-inflammatory & analgesic agents, respectively, along with polyphenols which were identified as antioxidant.^[13] Its anti-arthritis activity is yet to be explored but from the phytochemical constituents activity in extracts of *A.webbiana* makes it a prime candidate that might prove as a potent entity for treatment of Rheumatoid arthritis.

Materials and Methods

Procurement and authentication of plant material

Leaves of *A.webbiana* were procured from D. G. Ayurvedic Sangrah, Andheri, Mumbai, India., in September of 2019 and were identified & authenticated at The Patidar Jin Science College, Bardoli, Surat, Gujarat., by Dr. B. R. Patel ,Associate Professor of Botany, on 25th of September 2019. Authentication No. (Authen. /03/2019 Botany).

Drugs and reagents

CFA (Sigma Aldrich, Bangalore), methotrexate (locally procured from pharmacy store), methanol, tween-80, formalin and all other solvents and reagents utilized in study were of analytical grade and procured from authentic vendors.

Experimental animals and approval

Male wistar albino rats (200-250g) were obtained from Jai Research Foundation, Vapi, Gujarat, India. Animals were kept in specialized room having 25±1 °C temperature with relative humidity of 45-55% under 12 hr dark and 12 hr light cycle. Animals were allowed full access over food pellets and water *ad libitum*. Institutional Animal Ethics Committee (IAEC) approved the experimental protocol in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA), India (resolution no. CPCSEA/SNLPCP/IAEC/19/12/111) on 18th of January 2020.

Preparation of plant Extract:^[14]

A.webbiana leaves were powdered and subjected to extraction with methanol in Soxhlet extraction apparatus. Solvent was removed from extract under vacuum to

get semisolid mass. The extracted mass of *A.webbiana* was stored in vacuum desiccator (31.5% w/w) was used for experiments after suspending it in normal saline (0.9% NaCl) with 2% Tween-80 in specific doses to be administered by oral route. (MEAW – Methanolic Extract of *A.webbiana*)

Complete Freund's Adjuvant-induced Arthritis:[15]

Arthritis was induced by single sub-plantar injection of 0.1ml CFA (each millilitre contains 1mg of *Mycobacterium tuberculosis* in water in oil emulsion) into left hind paw of rats on day 0 in all groups except group 1. Treatment with MEAW and Methotrexate was commenced on 8th day after CFA induction in a manner of single dose a day for 20 successive days. All animals were euthanized on day 28th to evaluation of specific parameters.

- ⇒ Animals were isolated into six groups each containing six animals (n=6):
- Group 1 – Normal control;
 - Group 2 – Arthritic control;
 - Group 3 – Standard control (methotrexate 0.5mgkg⁻¹ p.o.;^[2]
 - Group 4 – MEAW 100mgkg⁻¹ p.o.;
 - Group 5 – MEAW 200mgkg⁻¹ p.o.;
 - Group 6 – MEAW 400mgkg⁻¹ p.o.;

Anti-arthritic potential of MEAW was characterized on injected paw on parameters of body weight and paw volume on specific days of 0, 7, 14, 21 & 28. On day 28th animals were euthanized and blood was withdrawn by retro-orbital puncture for characterization of haematological & serum biochemical parameters along with isolation of liver and injected paw for evaluation of anti-oxidant parameter and histopathological analysis, respectively.

⇒ Parameters evaluated:

- Measurement of body weight:
Body weight measurement was carried out on day 0 just before CFA induction and measured on days 7, 14, 21 & 28.
- Measurement of paw volume:
Paw volume was measured with the aid of plethysmometer (UGO Basile, Italy) on day 0 prior induction of CFA and on days 7, 14, 21 & 28. The difference between initial and final paw volume were calculated to get the change in paw volume.
- Arthritic index:^[16]
The visual arthritic index was used to evaluated the severity of arthritic as described (S. Wang et al., 2016).
- Haematological and serum parameters:
On day 28, haematological parameters like haemoglobin (Hb), red blood corpuscles (RBC), white blood cells (WBC), platelets (PLT) , erythrocyte sedimentation rate (ESR), c-reactive protein (CRP) & rheumatoid factor (RF) levels were measured.
- Biochemical parameters:
On day 28, biochemical parameters; aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) & total protein levels were measured.
- Anti-oxidant parameters:

Euthanized animals liver were isolated and washed with ice-cold saline. Tissue homogenates were formed with 0.1M HCl buffer. Supernatant was used to evaluate SOD ^[17], GSH ^[17] & MDA ^[18] levels as per assay methods demonstrated in respective articles.

- Histopathological analysis:

On day 28, injected hind paw ankle joints were isolated and placed in 10% formalin solution for 24 hrs following decalcification with 5% formic acid, prepared for paraffin embedding section at 5 μ thickness and stained with haematoxylin-eosin agent and evaluated under compound microscope with 40x magnifications for the existence of inflammatory cells infiltration, pannus formation, joint destruction.^[15]

Statistical analysis

Statistical analysis was done by the aid of Prism GraphPad 8.0 and data were expressed as mean $\pm$ SEM by utilizing one-way ANOVA with Dunnett's multiple comparison test. $p < 0.05$, $p < 0.01$ & $p < 0.001$ were considered significant, moderately significant & extremely significant, respectively.

Results

Body weight

Arthritic control rats demonstrated gradual depletion in body weight throughout the study compared to MEAW & Methotrexate administered groups. On day 28th, MEAW treated rats body weight of 400mgkg⁻¹ (254 $\pm$ 0.7) was evident in comparison with arthritic control group (238 $\pm$ 0.5). The data designated that MEAW increased body weight by 1.6% whereas Methotrexate increased body weight by 2.4% compared to arthritic control rats. (Fig- 1)

Paw volume

All CFA treated groups showed significant ($p < 0.001$) increase in paw volume in comparison with healthy control group. Methotrexate and MEAW 400mgkg⁻¹ showed extremely significant results ($p < 0.001$) by decreasing paw volume from day 14 onwards compared to arthritic control. MEAW lower doses 100 and 200mgkg⁻¹ showed dose dependent activity and lowered paw volume but were less efficient compared to MEAW 400mgkg⁻¹ and Methotrexate. MEAW 400mgkg⁻¹ (2.29 $\pm$ 0.009) was evident in comparison with arthritic control group (4.45 $\pm$ 0.008). (Fig-2)

Arthritic index

Arthritic index score surged after induction of CFA in rats compared with arthritic control group. Although, Methotrexate and MEAW 400mgkg⁻¹ resulted in significant ($p < 0.001$) depletion in arthritic score compared to arthritic control group. MEAW 400mgkg⁻¹ (2 $\pm$ 0.2) was evident in comparison with arthritic control (4 $\pm$ 0). (Table-1 & fig.3)

Haematological parameters

The surged levels of ESR, WBC & Platelets and depletion of Hb, RBC levels in arthritic control group were seen. whereas, in treatment groups, these conditions were reversed in dose dependent manner in MEAW groups significantly ($p < 0.001$) & Methotrexate showed significant ($p < 0.001$) results in regulating altered levels of these parameters. Along with this, CRP and RF levels in arthritic control which observed to be increased were decreased with treatment of MEAW and Methotrexate. (Table-2 & 3, fig 4,5)

Biochemical parameters

AST, ALT & ALP serum levels were increased significantly ($p < 0.001$) in all CFA induced groups along with total protein levels which were depleted significantly ($p < 0.001$) compared to healthy control. Methotrexate & MEAW treatment groups showed significant ($p < 0.001$) results in decreasing AST, ALT & ALP levels and total protein levels were increased significantly ($p < 0.001$). MEAW group effects were seen to be dose dependent. (Table-4)

Anti-oxidant parameters

GSH and SOD levels observed to be depleted in arthritic control group significantly ($p < 0.001$) in comparison with healthy control group. MEAW (dose dependent) and Methotrexate treatment resulted in significant ($p < 0.001$) increased in GSH and SOD levels compared to arthritic control group. MDA levels were increased in arthritic control group as compared to healthy control which too were regulated by MEAW treatment groups and Methotrexate significantly ($p < 0.001$). (Table-5)

Histopathological analysis

Histopathological findings demonstrated no inflammation in healthy control rats injected ankle joints with unharmed synovium along with zero bone necrosis (Fig-A). While arthritic control rats resulted in severe infiltration of inflammatory cells, altered synovium, bone necrosis (Fig-B). On the contrary, MEAW treated rats of 100, 200 & 400mgkg⁻¹ demonstrated significant prevention of bone necrosis and synovium tissue damage in dose dependent manner. MEAW 400mgkg⁻¹ demonstrated results next to Methotrexate with maximum protection against bone necrosis with fewer influx of inflammatory cells (Fig-F & C, respectively). MEAW 200mgkg⁻¹ showed moderate prevention with low levels of inflammatory cells infiltration (Fig-E). MEAW 100mgkg⁻¹ illustrated least protection against CFA induction with chronic presence of inflammatory cells along with damage to synovium and bone (Fig-D)

Discussion

The typical preclinical experimental model of RA includes adjuvant-induced arthritis model, since it possesses short duration of testing, easy measurement and similarities to human RA.^[19] Wistar albino rats were used for the study as it shares resemblance with human rheumatoid arthritis. The present study states

the impact of MEAW in CFA induced arthritis in rats, where MEAW 400mgkg⁻¹ demonstrated significant decrease of anti-arthritic effect in all inflammatory parameters.

The study indicated loss in weight of the arthritic rats relative to healthy control. This could be attributed to increment in production of leptin (a cytokine hormone) on administration of complete Freund's adjuvant which may also lead to reduced feed intake and hence weight loss.^[20]

Rat paw swelling (paw volume) used in assessing the degree of inflammation and the therapeutic effects of MEAW in this study reduced in volume. This may be attributed to immunological protection provided by the plants extracts, preventing systemic spread and ultimately reducing joint destruction in the rats. Reports have shown that prolonged inflammation is associated with the provocation of number of substances which initiate and moderate inflammatory response such as cytokines, granulocyte macrophage colony stimulating factor (GM-CSF), interferons and prostaglandins F (PGDF) which are connected with pain and degeneration of cartilage and bone that can culminate in severe impairment.^[21]

Arthritic index is a method to evaluate anti-arthritic activity of different drugs. To characterize the severity of arthritis, visual arthritic index was utilized.^[22] The scoring of arthritic index is simple, sensitive and real quick method for characterizing and assessing the degree of swelling and the therapeutic and the curative effects of drugs.^[23] From this study, it's evident that MEAW illustrated dose dependent reduction in paw swelling when compared to CFA induced arthritic control rats. Furthermore, histopathological analysis demonstrated protective effects of MEAW.

Reduction in Hb count in arthritis results from reduced erythropoietin levels, a decreased response of the bone marrow erythropoietin and premature destruction of red blood cells causes decreased levels of RBC in arthritic cases.^[24] In this study, treatment from MEAW and Methotrexate considerably increased Hb and RBC levels. A moderate rise in the WBC count occurs in arthritic conditions due to an IL-1 mediated rise in the respective colony stimulating factors cascading numerous cytokines.^[24] Where MEAW treatment demonstrated reduction in WBC levels to normal range along with hematological alteration of increased platelets levels were also restored.

The study indicated increase in the concentration of ESR in the arthritic rats relative to the normal control rats. This probably might be due to rise in the rate of making of tissue protein such as fibrinogen and α , β -globulin, and this elevation in the level of ESR is suggestive of serious but vague disease process. The acute phase protein (APP) in ESR exhibit similar features of presenting rise in the level in reaction to stress or inflammation such as injection, surgery, wound and death of tissues.^[25] From the study, treatment of the arthritic rats with MEAW showed reduction of ESR. The ability of MEAW to reduce the ESR may be attributed to its phytochemical content such as flavonoid because such phytochemical has been implicated in modulation of proinflammatory gene expression, and as such may cause diminution of swollen joints.^[26]

The level of CRP serves as indices of inflammation. CRP induces the release of inflammatory cytokines such as IL-1b, IL-6 and TNF-a by monocyte and may also directly function as proinflammatory boost to phagocytic cells.^[27] In this study, treatment of the arthritic rats with MEAW reduced serum CRP as compared with the untreated arthritic rats. Similar reports have shown that the level of CRP in the blood had direct relationship with RA development and gravity of the disease akin to RF.^[28]

Serum RF correlates with the quantity of serum IgM^[29] and the quantity of RF in the serum have direct link with the development of inflammation.^[23,30] In this study, the marked increase in the concentration of rheumatoid factor in the arthritic rats significantly reduced upon treatment with MEAW. This showed that the extracts exhibited anti-arthritic activity and the mechanism could be attributed to the making of autoantibodies towards the Fc fragment thereby shielding the breakdown of cartilage.^[31,32]

Studies have proved that serum AST and ALT has predominant role in the formation of chemical mediators such as bradykinins in inflammatory process, the protective effect of MEAW might be due to the presence of phytoconstituents providing antioxidants which further prevents lipid peroxidation^[33] and decreases the levels of cytokines.^[34] Serum total protein levels is lowered during inflammation, reflecting the changes in synthesis of this protein secondary to activation of hepatic cells by inflammatory cytokines, mainly IL-1. The rise in serum total protein levels by Methotrexate suggests stabilization of endoplasmic reticulum leading to protein synthesis or may be through MTX inhibition of IL-1.

GSH reflect the endogenous defense against damage caused by ROS (Reactive oxygen species) and organic peroxides as they act as an intracellular reductant in oxidation-reduction processes. The decreased levels of GSH in liver of arthritic rats might be due to the excessive consumption of GSH by the system to defend oxidative damage.^[35] The production of oxygen free radicals that occurs with the development of arthritis leads to decreased GSH and SOD levels as a consequence of their consumption during oxidative stress and cellular lysis,^[36,37] which is evident by decreased levels of GSH and SOD in arthritic control group.

The increase in lipid peroxidation maker, MDA in arthritic rats recorded in this study is suggestive of compromise in the antioxidant capacity and increased oxidative stress in RA.^[38] Lipid peroxidation is a critical mechanism of the injury that occurs during rheumatoid arthritis, which is often measured by analysis of tissue MDA. The large amount of MDA in arthritic control group is consistent with the occurrence of damage mediated by free radicals.^[39] MDA is biomarker that could furnish information on the overall lipid peroxidation level of a cell. Reports have shown that oxidative damage engendered by ROS is a crucial mechanism that underlies destructive and proliferative synovitis and articular degradation, and a substantial rise in ROS and H₂O₂ in arthritic rats is observed. Also, reduced antioxidant level is a risk factor of RA that aggravates the seriousness of the condition. The results of the histopathological observations substantiated biochemical findings as there was regeneration of the damaged epidermal layers as result of exposure to the adjuvant by treatment with the extracts. The effect of the extracts was comparable to that of Methotrexate.

Conclusion

The study revealed that MEAW possess anti-arthritic activity that is mediated by its anti-inflammatory effects on different parameters evaluated and also through various haematological, biochemical, anti-oxidant and histopathological parameters. All these results thus predict that MEAW provide pharmacological rationale for the traditional use of the plant against inflammatory conditions like rheumatoid arthritis.

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Table -1 Effect of MEAW on Arthritic index

Sr no.	Groups	Arthritic index
1	Control (0.1ml FCA + saline with Tween 80)	4 ± 0#
2	Standard (0.1ml FCA + MTX 0.5mg/kg, p.o)	1.83 ± 0.16***
3	MEAW 100 (0.1ml FCA + 100mg/kg, p.o)	2.83 ± 0.30**
4	MEAW 200 (0.1ml FCA + 200mg/kg, p.o)	2.33 ± 0.21***
5	MEAW 400 (0.1ml FCA + 400mg/kg, p.o)	2 ± 0.25***

The signs (***), (**) and (*) indicate values significantly different from control at P<0.001 (extremely significant), P<0.01 (moderately significant) and P<0.05 (significant) respectively. # indicates significant difference from normal control. * indicates significant difference from model control.

Table- 2 Effect of MEAW on haematological parameters

Sr no.	Groups	RBC (10 ⁶ cells/mm ³)	WBC (10 ³ cells/mm ³)	Hb(g/dL)	PLT (10 ³ cells/mm ³)
1	Normal	6.6±0.10	7.3±0.06	14.4±0.06	904±0.99
2	Control	3.5±0.07#	14.3±0.06#	9±0.04#	1733±1.54#
3	MTX	5±0.05***	8.4±0.05***	14.1±0.05***	1098±1.83***
4	MEAW100	3.7±0.04***	13.3±0.05***	10.6±0.04***	1328±1.63***
5	MEAW200	3.9±0.06***	11.6±0.07***	12.3±0.05***	1390±1.43***
6	MEAW400	4.7±0.06***	9.8±0.07***	13.3±0.07***	1191±1.06***

The signs (***), (**) and (*) indicate values significantly different from control at P<0.001 (extremely significant), P<0.01 (moderately significant) and P<0.05 (significant) respectively. # indicates significant difference from normal control. * indicates significant difference from model control.

Table- 3 Effect of MEAW on ESR, CRP and RF

Sr no.	Groups	ESR (mm/hr)	CRP (mg/L)	RF(IU/mL)
1	Normal	1.4±0.06	1.65±0.008	-
2	Control	7.8±0.008#	7.0±0.02#	57±1.07#
3	MTX	4.4±0.10***	2.8±0.013***	35±0.7***
4	MEAW100	5.6±0.09***	6.1±0.018***	51±1.3***
5	MEAW200	5.3±0.05***	5.1±0.02***	49±1.2***
6	MEAW400	4.8±0.04***	3.9±0.013***	41±1.1***

The signs (***), (**) and (*) indicate values significantly different from control at P<0.001 (extremely significant), P<0.01 (moderately significant) and P<0.05 (significant) respectively. # indicates significant difference from normal control. * indicates significant difference from model control.

Table- 4 Effect of MEAW on Biochemical parameters

Sr no.	Groups	AST(U/L)	ALT(U/L)	ALP(U/L)	Total Protein(g/dL)
1	Normal	42±0.055	42±0.557	71±0.966	6.5±0.04
2	Control	126±0.89#	179±1.20#	475±2.76#	5±0.10#
3	MTX	59±0.93***	53±0.98***	128±1.25***	6.4±0.04***
4	MEAW100	111±1.25***	169±1.16***	445±1.49***	5.2±0.03*
5	MEAW200	106±1.06***	124±0.01***	347±1.30***	5.5±0.06***
6	MEAW400	79±1.21***	69±1.11***	200±1.17***	6.1±0.08***

The signs (***), (**) and (*) indicate values significantly different from control at P<0.001 (extremely significant), P<0.01 (moderately significant) and P<0.05 (significant) respectively. # indicates significant difference from normal control. * indicates significant difference from model control.

Table- 5 Effect of MEAW on antioxidant parameters

Groups	SOD (units/mg protein)	GSH (µg GSH/mg protein)	MDA (nmol of MDA/mg protein)
Normal	4.4±0.115	75.1±0.44	2.1±0.04
Control	2.2±0.06#	43.8±0.70#	3.5±0.07#
MTX	3.5±0.07***	64.0±0.57***	2.7±0.06***
MEAW100	2.58±0.08*	51.5±0.61***	3.28±0.03*
MEAW200	3.0±0.03***	54.8±0.60***	3.2±0.036**
MEAW400	3.3±0.06***	62.6±0.71***	3.0±0.036***

The signs (***), (**) and (*) indicate values significantly different from control at P<0.001 (extremely significant), P<0.01 (moderately significant) and P<0.05 (significant) respectively. # indicates significant difference from normal control. * indicates significant difference from model control.

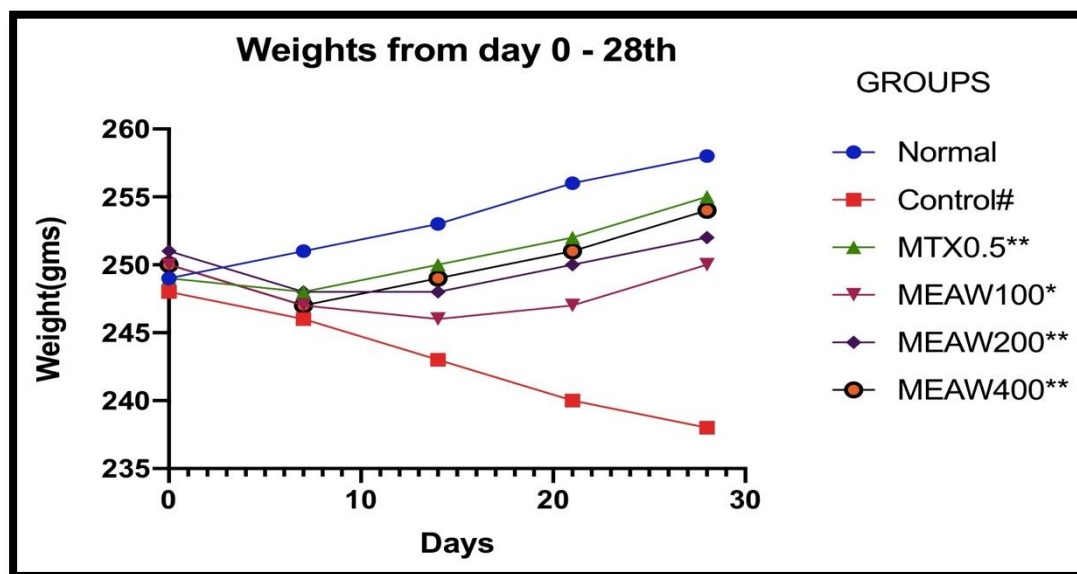


Fig. 1 Effect of MEAW on body weight

The signs (***), (**) and (*) indicate values significantly different from control at $P < 0.001$ (extremely significant), $P < 0.01$ (moderately significant) and $P < 0.05$ (significant) respectively. # indicates significant difference from normal control. * indicates significant difference from model control.

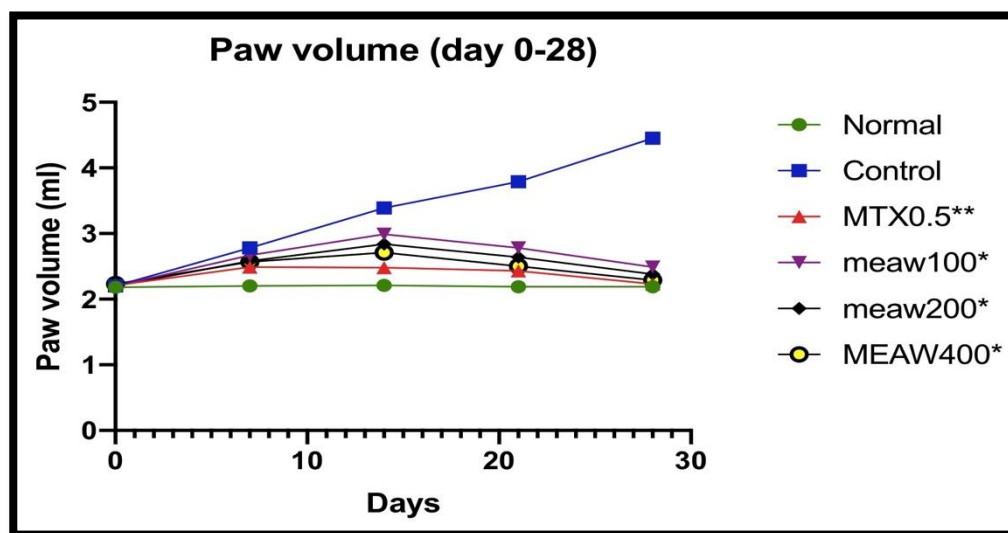


Fig. 2 Effect of MEAW on Paw volume

The signs (***), (**) and (*) indicate values significantly different from control at $P < 0.001$ (extremely significant), $P < 0.01$ (moderately significant) and $P < 0.05$ (significant) respectively. # indicates significant difference from normal control. * indicates significant difference from model control.

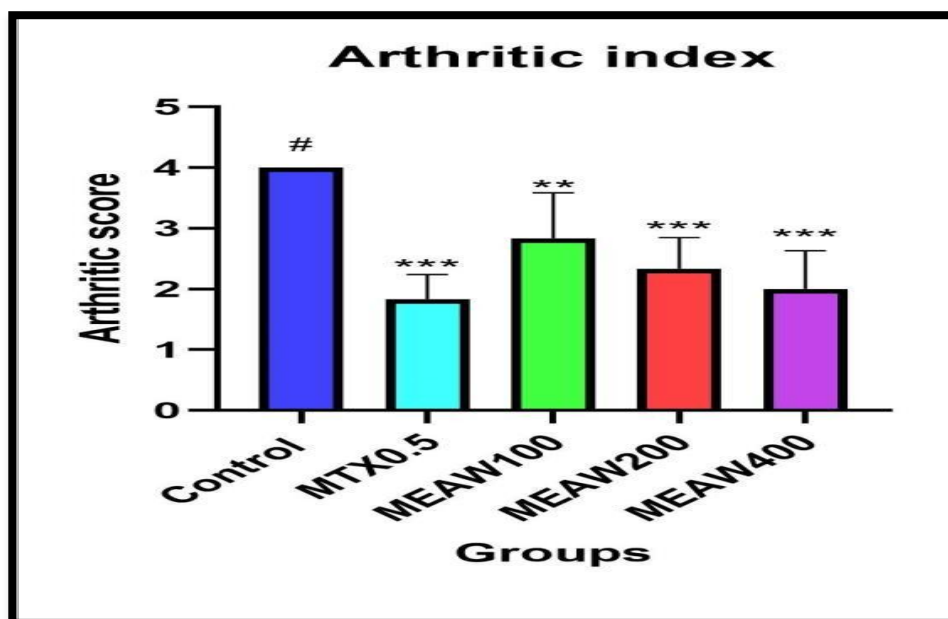


Fig. 3 Effect of MEAW on Arthritic Index

The signs (***), (**) and (*) indicate values significantly different from control at $P < 0.001$ (extremely significant), $P < 0.01$ (moderately significant) and $P < 0.05$ (significant) respectively. # indicates significant difference from normal control. * indicates significant difference from model control.

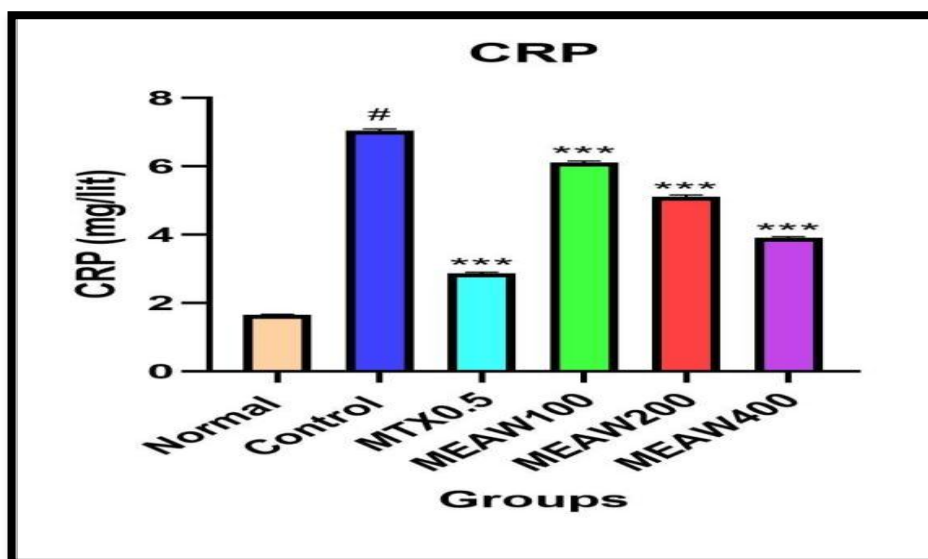


Fig. 4 Effect of MEAW on CRP

signs (***), (**) and (*) indicate values significantly different from control at $P < 0.001$ (extremely significant), $P < 0.01$ (moderately significant) and $P < 0.05$

(significant) respectively. # indicates significant difference from normal control. * indicates significant difference from model control.

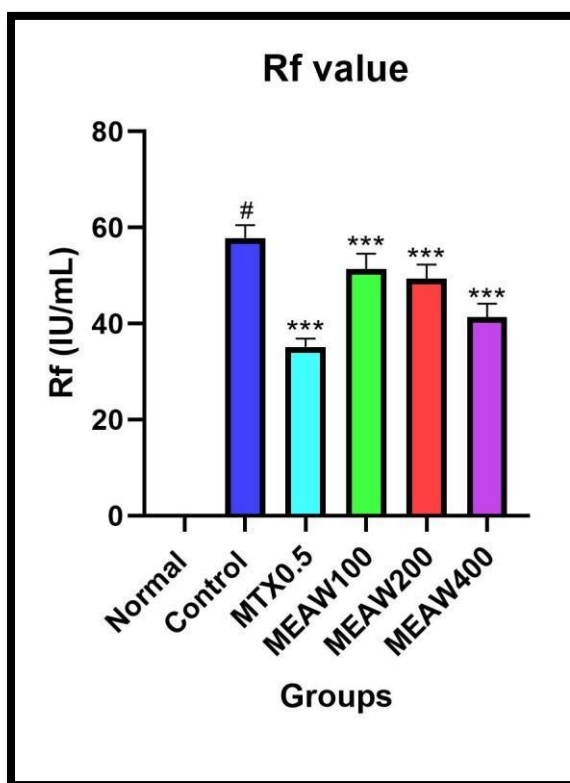
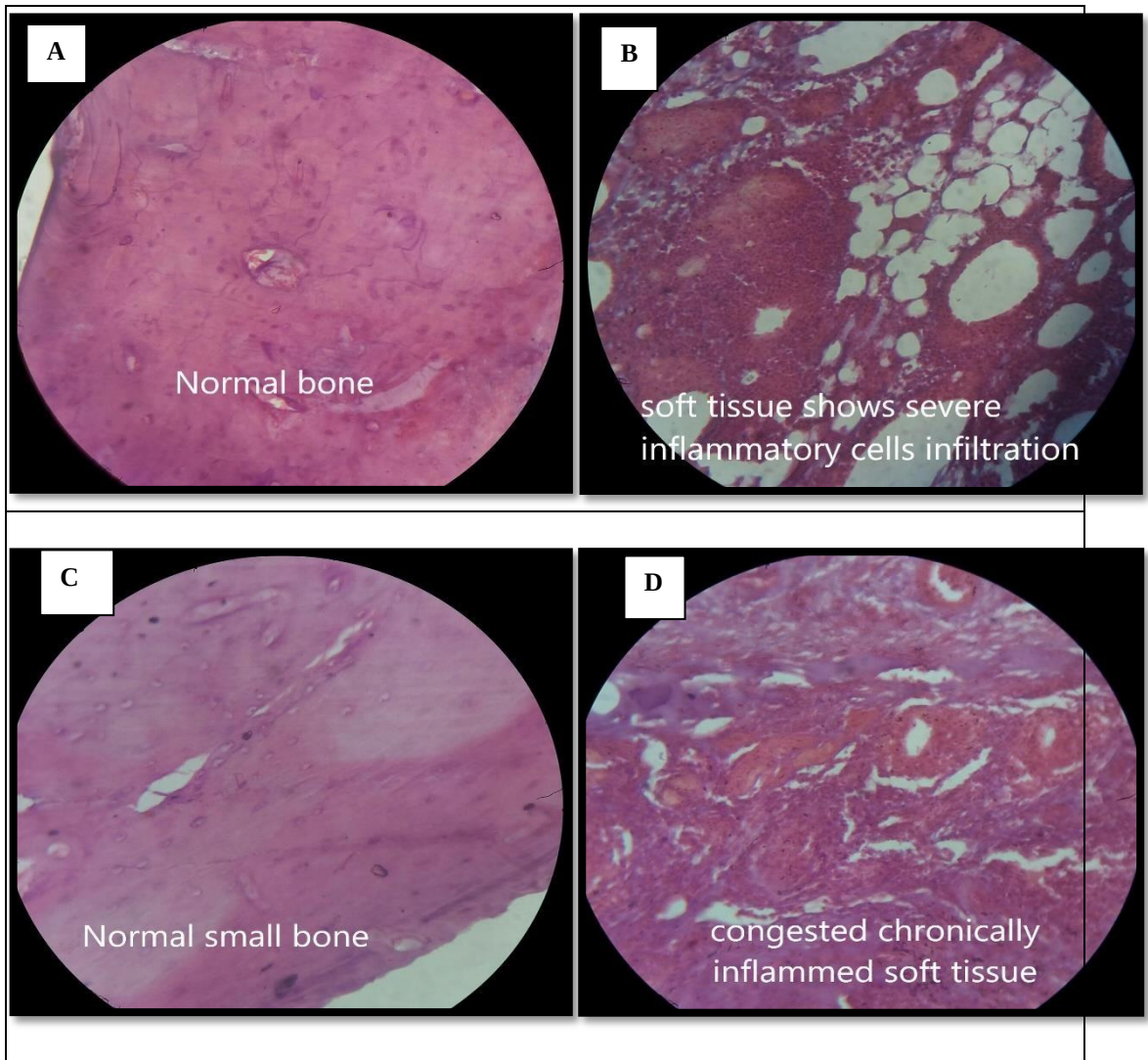


Fig. 5 Effect of MEAW on RF

signs (***), (**) and (*) indicate values significantly different from control at $P < 0.001$ (extremely significant), $P < 0.01$ (moderately significant) and $P < 0.05$ (significant) respectively. # indicates significant difference from normal control. * indicates significant difference from model control



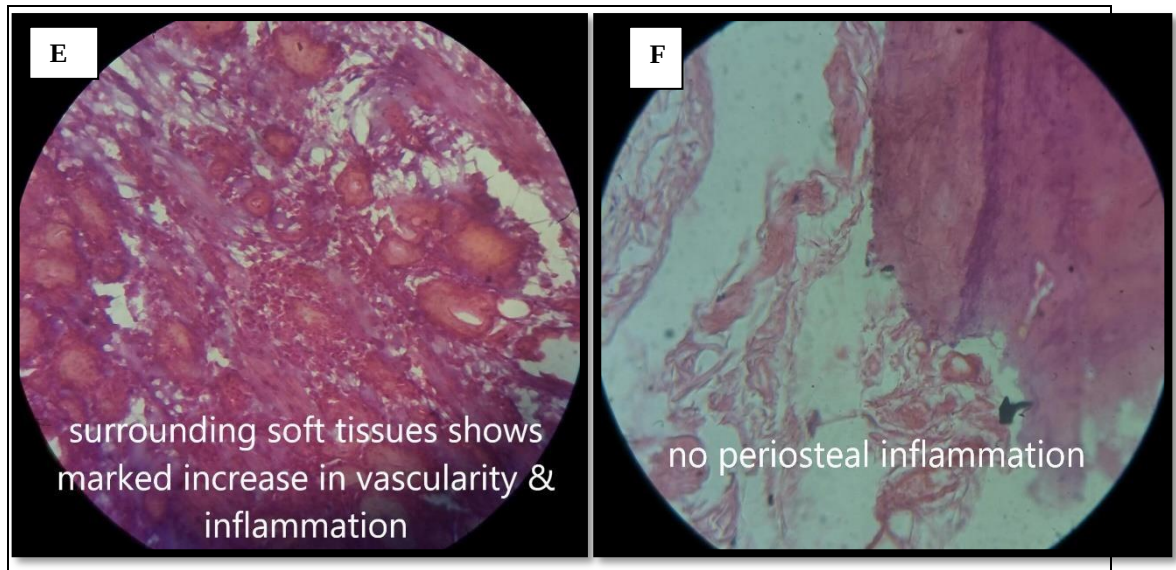


Fig. 6 Histopathology